Effects of 25-hydroxycholecalciferol (25(OH)D3) on broiler production performance and immune responses

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Introduction

Vitamin D3 (cholecalciferol) is generated within the skin of animals when exposed to ultraviolet light and is the inactive form of vitamin D. Due to modern indoor husbandry practices in commercial poultry production, vitamin D is an essential nutrient in broiler chicken diets.

Vitamin D3 is converted into its active form (1, 25(OH)2D3) following a two-step hydroxylation process using two key enzymes: 25-hydroxylase and 1α-hydroxylase (Figure 1). The active form plays a major role in calcium absorption by increasing its intestinal absorption and thereby supporting proper mineralization of bones in growing birds (Tatara, et al., 2012).

The first step in the biosynthesis of active vitamin D occurs mostly in the liver by the enzyme 25-hydroxylase, which hydroxylates cholecalciferol at 25-C position, to form 25-hydroxycholecalciferol (25(OH)D3) (van Etten, et al., 2008). In broiler chickens, dietary supplementation with 25(OH)D3 has increased efficacy, compared to vitamin D3 (cholecalciferol), in improving the bodyweight gain, feed efficiency, serum levels of 25(OH)D3 and decreasing the incidence of tibial dyschondroplasia (Atencio, et al., 2005; Yarger, et al., 1995). Even though the National Research Council (NRC) requirements for cholecalciferol is only 250 IU/kg feed, levels used in the poultry industry are quite high, reaching up to 4000 IU/kg feed, and most of the studies using 25(OH)D3 have been conducted under normal physiological conditions.
This research note focuses on understanding the effects of 25(OH)D₃ supplementation on broiler production performance and immune responses during an experimentally induced lipopolysaccharide (LPS) or coccidial challenge. LPS challenge model evaluates the animal’s ability to respond to an inflammatory stimulus while a coccidial challenge model generally uses anti-coccidial drugs to test the broiler’s capacity to recover from coccidiosis.

25-hydroxycholecalciferol supplementation improves bodyweight after coccidial and LPS challenges

Our current study addressed the effects of 25-hydroxycholecalciferol supplementation in broiler chickens under conditions of a mimicked bacterial infection using LPS. In our experiments, broiler birds were fed on a basal diet supplemented with either cholecalciferol (vitamin D₃), 25-hydroxycholecalciferol (25(OH)D₃) for the duration of the study or 25-hydroxycholecalciferol for the first 14 days and replaced with vitamin D₃ after 14 days. At 21 and 35 days old, birds were injected or not injected with lipopolysaccharide (LPS). At 24 hours post-LPS challenge, bodyweights were measured and bodyweight gain was expressed as pre-LPS bodyweight.

Similar to birds injected with LPS, birds challenged with live coccidial oocysts had improved bodyweight gain and decreased oocyst shedding post-coccidial challenge (Figure 3).

**Figure 2.** 24-hour bodyweight gain of birds in different experimental groups. Day-old birds were fed diets supplemented with either cholecalciferol (vitamin D₃), 25-hydroxycholecalciferol (25(OH)D₃) for the duration of the study or 25-hydroxycholecalciferol for the first 14 days and replaced with vitamin D₃ after 14 days. At 21 and 35 days old, birds were injected or not injected with lipopolysaccharide (LPS). At 24 hours post-LPS challenge, bodyweights were measured and bodyweight gain was expressed as pre-LPS bodyweight.

**Figure 3.** Effect of different doses of 25(OH)D₃ on bodyweight gain after coccidial challenge. Day-old birds were fed diets supplemented with 25(OH)D₃ at doses of 6.25, 25, 50 or 100 µg/kg of feed. At 21 days of age, birds were weighed individually and orally challenged with 1 x 10⁵ live coccidial oocysts. At 6 days after coccidial infection, bodyweights were measured and the percentage bodyweight gain over a 6 day period was expressed as pre-coccidial bodyweight. Bars (+ SEM) without a common superscript differ significantly within a group.
25-hydroxycholecalciferol supplementation improves bodyweight gain by altering the inflammatory responses

Inflammatory cytokines, such as IL-1β, IL-6 and tumor necrosis factor-α, are responsible for mediating a systemic acute phase response by the liver, which is characterized by anorexia, fever and decreased growth in animals (C.Klasing, 2004; Johnson, 1997; Johnson, et al., 1993; Klasing, 1998). On the other hand, anti-inflammatory cytokines like IL-10 suppress inflammation and improve bodyweight gain post-infection.

In parallel to increased bodyweight gain, birds supplemented with 25(OH)D3 and challenged with coccidiosis had lower IL-1β, and higher IL-10 post-coccidial challenge.

Figure 4. Effect of different doses of 25(OH)D treatment on (A) IL-1β and (B) IL-10 mRNA amounts in cecal tonsils. Six days after coccidial challenge, cecal tonsils were collected and IL-1β and IL-10 mRNA amounts were analyzed by real-time polymerase chain reaction (PCR) and normalized to β-actin and compared with the group fed 6.25 µg/kg of 25(OH)D3 and not infected with coccidia, so that all bars represent fold change compared to that group. Bars (+ SEM) without a common superscript differ significantly within a group.
25-hydroxycholecalciferol supplementation induces alteration in the inflammatory response leading to improved coccidial clearance

In conclusion, feeding 25(OH)D3 can be beneficial as it supports bodyweight gain and performance during health challenges frequently seen in commercial poultry production. During an infection, inflammatory cytokines can decrease bodyweight gain by decreasing feed intake. 25(OH)D3 supplementation prevents the negative effects of inflammatory cytokines on bodyweight gain by increasing the response of anti-inflammatory cytokines, without compromising the bird’s ability to fight the infection.

References available upon request